

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT :Lütticken et al.
SERIAL No. :USSN 09/084,837
FILED :May 26, 1998
FOR :RECOMBINANT BIRNAVIRUS VACCINE

EXAMINER :Mary E. Mosher
GROUP ART UNIT :1648

DECLARATION under Rule 132

Hon. Commissioner of
Patents and Trademarks
Washington, D.C. 20231

SIR

I, Egbert Mundt, residing at Dorfstrasse 37, D-18461 Millienhagen, Germany and one of the inventors of the above-mentioned US patent application declare as follows:

1
I have prepared the two recombinant IBDV constructs disclosed in Example 1 of the above-mentioned US patent application, designated IBDV/VP5⁻ and IBDV/VP5⁻-2. The two IBDV mutants were examined for their genetic stability as outlined below.

2
Serial passages of the two IBDV mutants were performed on primary chicken embryo cells (CEC). Each passage was screened for the occurrence of VP5 by using polyclonal rabbit anti-VP5 serum. The presence of virus was verified by using polyclonal rabbit anti-IBDV serum. In case of VP5-positive signals passaging was stopped.

Passage	IBDV/VP5 ⁻						IBDV/VP5 ⁻ 2	
	rabbit anti- VP5			Rabbit anti- IBDV			Rabbit anti- VP5	rabbit anti- IBDV
	1st. Exp.	2nd. Exp.	3rd. Exp.	1st. Exp.	2nd. Exp.	3rd. Exp.	1st. Exp.	1st. Exp.
1 st	-	-	-	+	+	+	-	+
2 nd	-	-	-	+	+	+	-	+
3 rd	-	-	-	+	+	+	-	+
4 th	-	-	-	+	+	+	-	+
5 th	-	-	-	+	+	+	-	+
6 th	+	-	+	+	+	+	-	+
7 th	n.d.	-	+	n.d.	+	+	-	+
8 th		+	n.d.		+	n.d.	-	+
9 th		n.d.			n.d.		-	+
10-18							-	+

These experiments demonstrate that the IBDV/VP5⁻ mutant is not stable upon increased passaging in cell culture whereas the IBDV/VP5⁻-2 mutant proved to be genetically stable. The IBDV/VP5⁻-2 mutant did not revert back to its wild type VP5⁺ phenotype at high passage levels.

3

All statements made herein of my knowledge are true and all statements made on information and belief are believed to be true; these statements are made with knowledge that wilful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 USC and that such wilful false statement may jeopardise the validity of the instant patent application or any patent issuing thereon.

Egbert Mundt

Egbert Mundt

Date : 24.11.2000

Signed at: *Sand Romy*, Germany